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14. ABSTRACT
More effective therapies for Myelodysplastic syndromes (MDS) can be developed if we learn more about how the disease develops. One of the most exciting advances has been the identification of mutations in genes encoding splicing factors. These occur in up to 85% of all patients with MDS. This group of proteins acts as a team to process the instructions (messenger RNA) that lead to the production of a specific protein. We have identified that the receptor for the most important growth factor for the production of granulocytes (the white blood cells most affected in MDS) is subject to splicing. These splicing changes result in a defective receptor, which fails to instruct blood cells to mature. We have developed a test to identify which specific splicing factor is involved in processing the messenger RNA for this receptor. We are identifying that specific splicing factor and are determining how to interrupt its defective splicing. Also, we have identified that this defective receptor results in too much growth and too little differentiation. We will develop a mouse model that will allow us to describe in greater, more accurate detail the molecular changes and cell behaviors due to the defective receptor. Our work will also allow us to screen for drugs that will correct the MDS condition by correcting the faulty splicing and may advance the use of the receptor as a clinical laboratory tool.

15. SUBJECT TERMS
Myelodysplastic Syndromes, Bone Marrow Failure, Granulopoiesis, RNA splicing

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INTRODUCTION: A major distinguishing feature of myelodysplastic syndromes (MDS), the most common form of acquired bone marrow failure, is the presence of recurrent mutations in one of the genes encoding a component of the splicing machinery. These mutations are found in 50-85% of individuals with MDS. However, little is known of their impact on normal and abnormal hematopoiesis. Our lab studies the signal transduction of Granulocyte Colony Stimulating Factor Receptor (GCSFR, the gene is *CSF3R*). The alternative splicing of *CSF3R*, which is associated with MDS, provides a robust model to reveal the mechanisms by which aberrant splicing promotes myelodysplasia and determine cell fate. We proposed the following specific aims:

Specific Aim 1. Determine the splicing mechanism involved in processing the *CSF3R* gene into transcripts encoding a full-length GCSFR and a truncation, differentiation-impaired GCSFR. We will construct a minigene reporter cassette and test the predicted mechanisms. We will determine which signaling pathways promote intron retention and permit expression of full-length GCSFR so to target this step pharmacologically.

Specific Aim 2. Fully characterize the aberrant proximal phosphoprotein and distal gene regulatory networks and correlate with an in vivo model of a truncated GCSFR. We will compare the signaling and gene expression profiles in murine and human CD34+ hematopoietic stem cells and correlate phenotypically with a retroviral transduction/transplantation model by expressing alternative splice form in the context of *Csf3r*$^{-/-}$ mice.

KEYWORDS: Myelodysplastic Syndromes, Bone Marrow Failure, Granulopoiesis, RNA splicing

ACCOMPLISHMENTS: We have made remarkable progress in aim 1, identifying the potential contribution of a tyrosine kinase, not Protein Kinase C, to intron retention (**Figure 1**). First, we improved the minigene construct, making it more specific for detecting spliced forms. Also shown in Figure 1 are data to suggest a possible role for U2AF1 in intron retention. In addition, we have obtained the cDNAs for other splicing genes: *SRSF2* and *Luc7L2*. A material transfers agreement for *SF3B1* has been signed. We will soon be performing site-directed mutagenesis to create the cDNAs for recurrent gene mutations associated with MDS. We have obtained pilot gene expression profile on Ba/F3 cell lines expressing either the full-length versus truncated CSF3R and have performed gene set enrichment analysis that documents different signatures for JAK-STAT, cell cycle, and cancer signaling. Thus, we are proceeding to breed the mice, transduce them with the alternatively spliced CSF3R, and perform the more informative RNA-Seq.

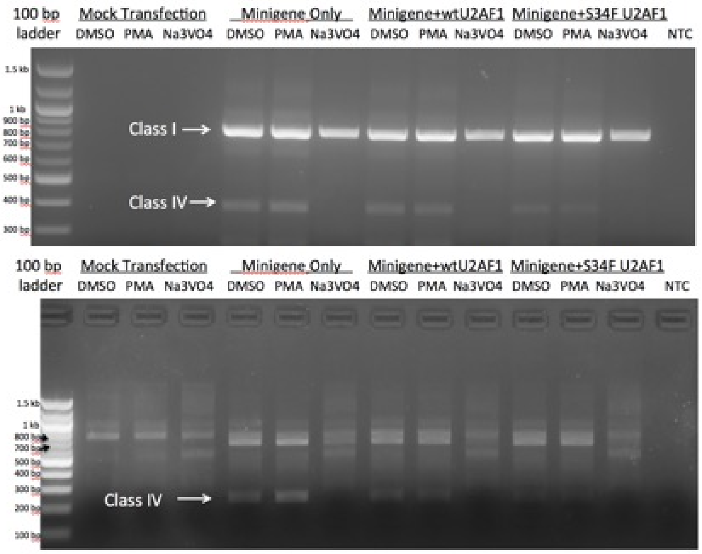

Figure 1. Effect of post-translational modification and U2AF1 on alternative splicing of CSF3R. 293 cells were transiently transfected with the *CSF3R* exon 15 minigene. RT-PCR was performed following 24 hr exposure of PMA (to activate Protein Kinase C) or sodium vanadate (to inhibit tyrosine phosphatases). Cells were also co-transfected with wild-type or mutant *U2AF1*. Absence of bands in vanadate or U2AF1 S34F conditions suggest intron excision, which results in alternative splicing of *CSF3R.* NTC, no template control. DMSO is a diluent control

IMPACT: We have tentatively identified U2AF1 and tyrosine phosphorylation as a splicing factor and post-translational modification that regulate the processing of the CSF3R transcript. This will identify a pathway for therapeutic targeting in MDS. We submitted an abstract on this work, which was accepted for presentation at the American Society of Pediatric Hematology/Oncology and American Society of Hematology annual meetings in 2016.

CHANGES/PROBLEMS: Delay in obtaining institutional release from Northwestern University School of Medicine has resulted in delay of the start-up. However, the award has been transferred and animal care approval has been obtained at VCU. Also, the co-PI Chonghui Cheng, MD PhD has moved from Northwestern to Baylor College of Medicine. The transfer and administrative paperwork has been successfully completed. I have successfully recruited a doctoral student, Ann Wang, to work on this project.

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